

FACILITIES AND OTHER RESOURCES - CHODERA LABORATORY

Computer: All lab members are equipped with laptop computers with integrated graphics processors (GPUs), and have access to high-performance development machines containing a range of modern GPU accelerators. The group has priority access to a high-performance computing cluster with 1920 total hyperthreads and 120 NVIDIA GTX-680, GTX-TITAN, or GTX-TITAN-X GPUs. Project storage is provided by a high-performance shared 1.5PB GPFS storage system. Dedicated servers provide access to Folding@Home, which currently provides ~19 PFLOP/s aggregate computational power in over 350,000 actively computing cores—**equivalent computing facilities would cost tens of millions of dollars**. Network connections are at least 1 Gbit/s throughout MSKCC, with HPC systems connected at 10 Gbit/s.

Laboratory: The Chodera wetlab occupies ~340 square feet of space. The central feature of the wetlab is an integrated platform for fully automated biophysical experiments instrumented for remote monitoring and operation. This system includes a Thermo BenchTrak Orbitor, a Tecan EVO200 with three dispensing technologies (including an HP D300), four Inheco incubators, a BioNex HiG4 centrifuge, Tecan Infinite M1000PRO plate reader (capable of absorbance, fluorescence and FP, luminescence, and AlphaScreen measurements, with injectors installed for kinetics measurements), Caliper GXII microfluidic electrophoresis platform, Roche LC480 qPCR machine, Agilent VCode barcode printer and PlateLoc plate sealer, Thermo MultiDrop Combi reagent dispenser, and Thermo automated Cytomat Hotel. **This platform automates cloning, site-directed mutagenesis, recombinant bacterial protein expression and purification, cell-free transcription and translation, microfluidic gel electrophoresis, Thermofluor protein stability assays, and fluorescence measurements of binding affinities. It can also automate preparation of ITC and SPR experiments that can be conducted at the Rockefeller HTSRC across the street.** There is bench space for one group member to work manually using standard molecular biology tools. A Mettler-Toledo Quantos automated gravimetric solution preparation system ensures compound concentrations are always accurately and traceably prepared. An electronic lab notebook tracks all materials and measurements in the laboratory using barcodes. Shared equipment space, standard laboratory refrigerators and freezers, and common shared equipment (centrifuges, incubators, etc.) is also provided. Both experimental and computational spaces are located in Memorial Sloan-Ketterings new Zuckerman Research Center (ZRC).

Animal: N/A

Office: All lab members have desks in a modern open-plan computational biology working space where the Chodera lab currently occupies ~400 ft². Group members are equipped with monitors, backup storage, and other standard workstation accessories. Additional office space includes Dr. Chodera's office, office space for a shared administrative assistant, shared conference rooms, and meeting and library space.

Clinical: N/A

Other Resources:

The Rockefeller high-throughput screening resource center (HTSRC) is located across the street at the Rockefeller University. The HTSRC provides a number of high-throughput binding and biophysical measurement facilities at a minimal cost to us, most notably (1) a GE/MicroCal Auto-iTC200 automated isothermal titration calorimeter capable of processing up to 384 samples unattended, and (2) a Proteon XPR36 SPR instrument (capable of processing 96 samples), among others.

The MSKCC Analytical NMR Core under the direction of Dr. George Sukenik allows for the unattended 1H-NMR characterization of compounds using an automated sample workflow.

Numerous additional MSKCC core facilities are available to MSKCC researchers, including proteomics and mass spectroscopy, NMR, X-ray crystallography, high-throughput screening, analytical chemistry, DNA sequencing, and bioinformatics consulting. Many of these core facilities are highly automated. Over 30 core facilities are currently available, all directed by Ph.D.-level experts available for consultation.

FACILITIES AND OTHER RESOURCES – SEELIGER LABORATORY

Environment – Contribution to Success

The facilities and other resources available to the PI, Markus Seeliger and his research team at Stony Brook University include everything needed to undertake and complete the proposed research project successfully. The intellectual environment in Stony Brook and the nearby New York area is excellent. The following investigators are located in the environment of the PI and perform complementary research. *They dramatically increase the likelihood of success for the project by providing constructive criticism and informal intellectual input.*

INVESTIGATOR	DEPARTMENT	EXPERTISE
Yibing Shan	DE Shaw Research (NYC)	Microsecond timescale MD simulations of protein drug interactions.
Ken Dill	Laufer Center (LC) for Physical and Quantitative Biology	Mathematical modeling and ligand docking methodology.
Jian Cao	Medicine	Targeting metastasis for anti-cancer therapy
Evangelos Coutsiias	Applied Mathematics and Statistics / LC	Computational approaches to geometry and conformation of macrocyclic compounds.
Miguel Garcia-Diaz	Pharmacology	Structural biology of protein-DNA complexes.
Steven Glynn	Biochemistry	Structural biology of proteases.
John Haley	Pathology	Drug Discovery of anti-cancer therapeutics, kinase signaling, medicinal chemistry.
Yusuf Hannun	Cancer Center	Lipid mediators of cancer cell signaling
Dmytro Kozakov	Applied Mathematics and Statistics / LC	Computational approaches to ligand docking, PPI and macrocycle – protein interaction.
Todd Miller	Biophysics/Physiology	Biochemistry of tyrosine kinases.
Lina Obeid	Cancer Center	Sphingolipid signaling in cancer.
Iwao Ojima	Chemistry	Medicinal Chemistry / Anti-cancer drugs
Daniel Raleigh	Chemistry	Biophysics/NMR of protein folding and protein/protein association/aggregation. Provides access to biophysical equipment for the project.
Robert Rizzo	Laufer Center / Math	Computational ligand docking methods.
Carlos Simmerling	Laufer Center / Chemistry	MD force field development.
Peter Tong	Chemistry	Mechanism of enzyme inhibitors with slow onset kinetics / Medicinal Chemistry / Drug Discovery
Jin Wang	Chemistry	Method development for computational ligand binding predictions / calculation of ligand binding energy landscapes.

Particular highlights that contribute to the success of the project are:

- New 5000 sq ft laboratory space shared by three structural biology groups opened July 2012.
- New center for computational and quantitative biology (Laufer Center) opened in May 2012.
- Renovated structural biology center with the latest state-of-the-art NMR instrumentation (new 700 MHz and 850 MHz instruments) and robotics for X-ray crystallography.
- Local access to Brookhaven National Laboratory X-ray beamlines and home of the latest synchrotron X-ray source (NSLS-II) in the world from 2015.
- SPR instrument (GE Biacore T200), purchased with NIH-supported instrumentation grant (Seeliger, PI) with increased sensitivity and throughput to allow drug binding studies to immobilized proteins.

Institutional Commitment to Dr. Seeliger (PI)

There is extensive evidence of institutional commitment to his development as an academic researcher. The PI has a salaried 12-month, tenure-track academic appointment in the School of Medicine, with a light teaching requirement of 20-24 contact hours per year of teaching. The start-up package provided to him includes laboratory space, equipment, and research funds needed for this first R01 application. Administrative support is provided to the PI by a departmental administrative core. Important career-development programs are also available to him, including development workshops and grantsmanship counseling offered by the Office of Faculty Development (School of Medicine); and a seminar series by and for department faculty. At one such seminar, the PI presented research ideas from this proposal and received feedback from his department colleagues that informed this application. The PI is also completed recently a 18 month faculty development program: "Leaders in Medical Education" and participated in a workshop on leadership in the life sciences at Cold Spring Harbor (2/2012). The PI regularly discusses scientific strategy and management issues with his mentoring committee which consists of faculty from the department of pharmacological sciences, biophysics and the Laufer Center.

The PI is also affiliated faculty in the recently opened Laufer Center for Physical and Quantitative Biology, which provides a stimulating academic environment for the biophysical work proposed here as well as video conferencing, conferencing and computational resources. His research falls under structural biology and cancer research, one of three university-wide priority research areas, which will help ensure continued institutional commitment to him and his research program.

Facilities:

Laboratory:

Markus Seeliger is assigned a newly built 1500 sq ft laboratory space that is located in the Basic Science Tower, Department of Pharmacological Sciences adjacent to his office. His space is located within a larger room shared with two other department faculty and comprises bench space for twelve researchers and equipment (plus 200 sq ft per additional full time employee). The following shared laboratory space is available adjacent to the central bench space: chromatography room (150 sq ft), fermentation room (250 sq ft), radioactive isotope work room (100 sq ft), cold room (120 sq ft), tissue culture room with two tissue culture hoods (200 sq ft), two equipment rooms (120 sq ft and 100 sq ft), temperature controlled crystallography room (140 sq ft), chemical work area with two chemical hoods (200 sq ft), and a computer office (122 sq ft).

Minor equipment (cost <\$5K) in his space includes: an electroporator for bacterial transformations; a microcentrifuge; DNA and protein gel electrophoresis and blotting equipment; digital water and dry baths; two analytical balances; pH meter; sonicator waterbath; one ultralow and one low temperature freezers; one refrigerator; and two incubator ovens. *These laboratory facilities were specifically designed and equipped to support the proposed research.*

Clinical: Not applicable.

Animal: Not applicable.

Computer:

Markus Seeliger uses a Lenovo ThinkPad X60 (Windows 7) and a MacBook Pro for office work. Each coworker is equipped with a similar laptop or desktop. The PI's lab has one Dell UNIX multi-core workstation for data processing. The lab also shares several PC computers driving equipment. Each bench space and equipment space is equipped with at least two hardwired broad band internet connections in addition to wireless internet across the entire laboratory. Computer servers, unlimited backup storage, color laser printing/copying and IT support are provided by dedicated departmental staff. The Laufer center provides access to multi-core clusters for larger scale ligand docking simulations (688 Intel Xenon CPU cores and 112 Nvidia Tesla GPUs, with a 40 TB NFS and 113 TB Lustre storage system). *The combination of these information technologies contributes to the potential for success by assuring efficient data handling and fidelity.*

Office:

Markus Seeliger's newly built office space is 190 sq. ft. and is located adjacent to his laboratory. It is equipped with office furniture, four hardwired high-speed internet access points and a phone line. The students'/technicians' desk space, which is located in the laboratory, has similar network access. Laboratory members also have unrestricted access to an adjacent conference room with projection equipment, whiteboards, and table space and chairs for 16 people. The departmental main office on the floor above provides access to FAX machines, color photocopiers, stationary and secretarial services. *These facilities assure that the PI and his immediate research team will have the necessary space in which to formulate experiments, analyze results, and prepare manuscripts for publication.*

Other Resources:

The Center for Structural Biology is located in the Center for Molecular Medicine building adjacent to the PI's laboratory and provides X-ray crystallography and NMR core facilities. The recently renovated X-ray crystallography core facility (approx. 500 sq ft) is temperature controlled and houses a 50 sq ft cold room. The center has been equipped in Spring 2012 with an integrated robotic crystallization suite (Formulatrix) consisting of two NT8 nanoliter dropsetter (one at 4 °C, one at 23 °C), Formulatrix 10 screen making robot and a RockImager 180 automated imaging microscope for crystal imaging and storage of crystallization experiments. All systems are networked and linked to an experiment database. All data are backed-up to the departmental backup server. The center operates two Rigaku X-ray diffractometers (RU-H3R 18 kilowatt generator, MSC confocal blue mirror optics and a Raxis IV detector) with controller computers. The following minor equipment is available in the crystallography suite: a SBS plate mixer/shaker, two Zeiss stereomicroscopes and three temperature controlled 6 ft incubators. The X-ray beamlines at the National Synchrotron Lightsource (NSLS) at Brookhaven National Laboratory (BNL) are less than 30 min away by car. Ample experiment time is available for X-ray crystallography and small angle X-ray diffraction data collection. Experienced staff at BNL provides outstanding training and informal intellectual input. The National Synchrotron Lightsource II (NSLSII) has achieved first light and start of operation for X-ray crystallography is anticipated for spring 2016. NSLSII will provide world class resources for X-ray crystallography including X-rays up to 10,000-times brighter than NSLS1 as well as microfocus beamlines. In addition to beamline access at NSLSII, we have access to other synchrotron sources around the country through collaboration agreements (e.g. CHESS, ALS, APS, etc).

The NMR instrumentation core is located in the center for structural biology and the chemistry department (5 min distance by foot, on-campus). A Bruker 850 MHz instrument with three channel high sensitivity cryogenic probe has been installed in the structural biology center in March 2013. A Bruker 700 MHz instrument with four channel high sensitivity cryogenic probe and a 500 MHz Bruker Instrument with a "Prodigy" cryogenic probe has been installed in January 2013 in the chemistry department (10 min walk, on-campus). The new instruments have multiyear warranties including cryogenic probes. The total NMR instrumentation available consists of Bruker 850 MHz with cryo probe, Bruker 700 MHz with cryo probe, Bruker 500 MHz with "Prodigy" cryo probe, a second Bruker 700 MHz with conventional probes, Bruker 600 MHz solid state, Bruker 500 MHz solid state, 400 MHz and 300 MHz instruments for small molecule work. Two PhD-level scientists with extensive experience in protein NMR operate the facility and train users. A computational core with hard- and software for NMR and X-ray data analysis is available. *Availability of the structural biology core contributes greatly to the potential success of this project, which includes NMR and X-ray crystallography as a core experimental methodology.*

Mass Spectroscopy: *The University Mass Spectroscopy Facility* is housed in the Chemistry department and in the School of Medicine. It operates instruments for electrospray, MALDI-TOF and FAB. The director of the facility, Dr Charles Iden has over 30 years of experience in biological mass spectroscopy as well as small molecule mass spectrometry. Of most relevance to this proposal are electrospray and MALDI-TOF instruments.

The Proteomics Center at the school of medicine is located in the same building as the PI's laboratory. The proteomics center is staffed by two Ph.D. level scientists and two staff scientists with long-term experience in biological and small molecule mass spectrometry. Of most relevance to this application are the Applied Biosystem Voyager DE-STR MALDI-TOF workstation for protein characterization, the Thermo Fisher Scientific

LTQ Orbitrap XL ETD for analysis of peptic digests and the Thermo Fisher Scientific TSQ Quantum Access triple quadrupole mass spectrometer for small molecule detection. The facility also operates a Biacore 2000 surface plasmon resonance instrument for the determination of ligand binding/dissociation kinetics. **We recently installed a new SPR instrument (T-200).** 24/7 access to the instrument will be guaranteed.

Computational Cluster: Stony Brook houses a local cluster for parallel computing (SeaWulf). The Seawulf Cluster is a custom-built 470-processor Linux Cluster. The cluster uses 3.4GHz Intel Pentium IV Xeon CPUs interconnected with Gigabit Ethernet. The data are stored on five IDE RAID storage systems, with a total of 20TB of high speed disk space. Individuals get unlimited access for 15 CPU (30 processors). Additional CPU time is arranged by informal applications. The Seeliger group has received on average 36,000 CPU hours per month for docking studies. Docking of each ligand requires an average of 7 min / CPU (= 0.3 million compounds per month using our allocation). Processing of docked results and rescoring of compounds requires an additional 10,000 CPU hours. Availability of the computer resources and the expertise in computational docking (Dr. Rizzo) greatly support the potential success of the project, which makes heavy use of the computational docking methodology.

The Genomics Core Facility on the 8th floor of the Health Science Tower has a complete Affymetrix microarray facility including bioinformatics services.

Imaging Lab:

The Microscopy Imaging Center is equipped with state-of-the-art imaging capacity. Equipment includes a Multiphoton Confocal Microscope and Digital Transmission Electron Microscope, both of which are jointly used by University Hospital Pathology Department for clinical sample analysis. Services include:

- 2-photon Confocal Microscope (User Operated)
- Transmission electron microscope (Technician-operated)
- Sample preparation and handling
- In vivo 2-photon microscope (Technician-operated)
- Commercial and in-house software for data analysis and image analysis
- SIM and STORM super-resolution microscopy (User- or technician-operated)
- Training and technical service are both available, as well as a range of support services.

The Center for the Analysis and Synthesis of Macromolecules: Located in the Department of Biophysics provides DNA sequencing and protein sequencing.

The Chemical Synthesis Center: provides custom synthesis on a fee for service basis.

Institute Of Chemical Biology & Drug Discovery provides a parallel organic synthesis facility and a screening facility; as well as bio-analytical instrumentation

Molecular Cloning Service:

- The facility core accepts subcloning and mutagenesis projects, which is especially helpful for labs with expertise in areas other than routine molecular biology. The core has >500 stock vectors including a variety of CRISP/Cas reagents and can provide consultation to assist with choosing the correct plasmid vector for a specific experimental goal and the design to generate the clone needed.

FACILITIES AND OTHER RESOURCES – GUNNER LABORATORY

Computer: All lab members are equipped with a personal computer. They all have access to the Gunner lab CPU with 192 cores.

Laboratory: N/A

Animals: N/A

Office.

The students and postdocs share ≈ 300 ft² of open office space. Group members have appropriate computers, monitors, printers and data backup.

Gunner, the PI has a separate office of ≈ 100 ft².

The offices are located in the CDI building on the CCNY campus. The group has access to shared conference rooms and spaces for eating and meeting.

Clinical: N/A